REMARKS

Upon entry of the present amendment, claims 6-10, 14-17, 27, and 28 will be pending. Applicants have amended claim 6 to specify that the recited cell is a mammalian cell in vitro. Support for these amendments can be found throughout the application as filed, e.g., at page 17, line 22, to page 21, line 23 (Examples 3 and 4), *inter alia*. No new matter has been added.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 6-10, 14-17, 27, and 28 were rejected as allegedly unclear for recitation of the phrase "lacking at least amino acids 495-517, 525-547, and 555-575 of the Zinc finger region of human Sp3" in claim 6.

While Applicants do not agree that this phrase is unclear, Applicants have amended claim 6 to change the format thereof for additional clarity, and to recite "wherein at least amino acids 495-517, 525-547, and 555-575 of the Sp3 Zinc finger region are lacking from said fragment of human Sp3," substantially as suggested by the Examiner.

In light of this amendment, Applicants request that the rejection under 35 U.S.C. § 112, Second Paragraph, be withdrawn.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 6-10, 14-17, 27, and 28 were rejected for allegedly failing to comply with the enablement requirement. Applicants respectfully traverse.

At page 5, the Office Action states that:

> ... It appears the only intended use of an agent identified by the claimed method is for an anti-proliferative or anticancer agent. The specification, however, does not provide a nexus between agents identified by the claimed two-hybrid system and their anti-proliferative and anticancer effects. Further, the specification does not provide a nexus between agents identified by the claimed two-hybrid system and their function in activating Sp3-mediated transcription in any cells either in vivo or in vitro.

Applicants submit that the Office is improperly reading in limitations from the specification that are not present in the claims. The pending claims are directed to methods of identifying agents

that activate TSA-responsive Sp3-mediated transcription, and thus are not limited solely to agents useful as anti-proliferative and anticancer agents. One of skill in the art would recognize that agents identified by the present methods may be candidate agents for modulating proliferation in a variety of contexts, either *in vivo* or *in vitro* as desired – the claimed methods are simply novel methods to identify agents that activate TSA-responsive Sp3-mediated transcription. While one use for such agents is disclosed in the specification (e.g., as an anti-proliferative or anticancer agent, see, e.g., page 4), others are also known in the art (e.g., as a pro-proliferative agent, as the Office pointed out at pages 7-9); therefore, it is improper to restrict the claims to methods of identifying agents useful as anti-proliferative and anticancer agents.

Furthermore, even if the claims were so limited (and they are not), there is no question that they still would be adequately enabled. The "nexus" that the Examiner appears to believe to be necessary is amply provided by the experiment in Example 3 of the specification. In this Example, Applicants demonstrated that Trichostatin A (TSA, an agent known to have anti-proliferative effects—see, e.g., page 1, lines 15-23) gives a positive readout in an assay utilizing the first and second vectors specified in claim 6, i.e., an assay of the invention. It is known that a downstream effect of TSA is activation of p21/WAF1/Cip1, a cyclin-CDK inhibitor (page 2, lines 11-13). Applicants showed that this anti-proliferative effect of TSA is mediated by TSA's effect on Sp3, and in particular on the transcription activation domain of Sp3. The present method is designed to capitalize on that observation, in order to identify other agents that, like TSA, can stimulate the transcription activation domain of Sp3. Such agents would be expected to function like TSA, i.e., to induce intact Sp3 to activate expression of p21/WAF1/Cip1. It is not clear to Applicants why the Examiner does not believe the experimental results disclosed in the application, or at least does not believe they constitute evidence that a compound that (like TSA) gives a positive readout in the claimed method is likely to function like TSA, and thus to have anti-proliferative activity. If the Examiner decides to maintain this aspect of the rejection, she is asked to address this point.

The Office Action refers in numerous places to "the claimed two-hybrid system." See, e.g., page 5, lines 16 and 18, and page 6, lines 9 and 11. This reflects a fundamental

misunderstanding of the claimed method that has apparently contributed to the Examiner's enablement concerns. Applicants are not claiming a method that specifies anything resembling a "two-hybrid system" as alleged by the Office. In fact, the present methods function quite differently than classic two-hybrid systems. In a two-hybrid system, the activation and DNA binding domains of a transcriptional activator (such as GAL4) are split into two different fusion proteins encoded by two different constructs. The activation domain is linked to a first heterologous protein in the first fusion protein, and the DNA binding domain is linked to a second heterologous protein in the second fusion protein. These two fusion proteins provide a means to study whether the first and second heterologous proteins associate with each other and thus can be considered "binding partners." If and only if the two heterologous proteins associate together, the activation and DNA binding domains of the transcriptional activator are brought together in sufficient proximity to form a functional unit that can induce transcription of a reporter gene on a third construct (this third construct bearing a sequence recognized by the DNA binding domain of the transcriptional activator). If the two heterologous proteins do not bind together or otherwise associate with each other, the transcriptional activation domain attached to one is not brought into proximity with the DNA binding domain attached to the other, so the two domains cannot cooperate to induce transcription of the reporter gene.

In contrast, the present methods specify use of a first vector that encodes, together in a single fusion protein, (1) a fragment of Sp3 that has transcriptional activation activity (e.g., the activation domain of Sp3), and (2) a DNA binding domain from a different (i.e., heterologous) protein. *Unlike a two-hybrid system*, the activation domain and the DNA binding domain used in the claimed methods are not from the same transcriptional activator protein. *Unlike a two-hybrid system*, these two domains are not split into separate fusion proteins, but rather are linked together in a single fusion protein. *Unlike a two-hybrid system*, the present fusion protein is not employed as a means to detect interactions between two different heterologous proteins present on separate fusion proteins. In fact, the sole resemblance between the presently claimed methods and a "two-hybrid" system is the use of a reporter vector bearing a target binding sequence for a transcriptional activator, or a DNA binding domain of a fusion protein, operably linked to a

reporter gene. This reporter vector is the "second vector" specified in the present claims, and corresponds to the "third construct" described above as used in a two-hybrid system. The reporter gene on this second vector bears no relationship to either of the hybrid genes utilized in a two-hybrid system. It is simply the reporter.

The distinction outlined above is important, as some of the Examiner's stated concerns derive from her mistaken belief that the claimed methods constitute a "two-hybrid" system and thus suffer from certain problems associated with such systems. For example, the Office action at pages 6-7 cites Allen et al. (TIBS, 1995, 20:511-516) as teaching that

> while the two-hybrid system is useful for studying protein-protein interactions via activation of reporter-gene expression, there are several limitations and problems, and its application is limited (p.512, col. 2). Allen et al specifically teach that the most critical consideration in performing two-hybrid screens is whether true positives isolated in the system are actually representative of *in vivo* cellular interactions. "It is possible to identify interacting partners that never associate in vivo because they are normally expressed in different cell types, localized in distinct cellular compartments, expressed at different developmental stages etc" (page 513, col. 1, last para).

Allen et al. are talking about a screen used to determine whether two particular proteins physically interact *in vivo*, as a means to examine their natural biological functions. The authors do not question whether the screen can identify proteins that are capable of interacting. That it can do so is understood. Instead the authors simply point out that evidence that two proteins are capable of interacting does not constitute evidence that they actually do so *in vivo*, as two interacting partners might be identified in the screen even though they are not naturally expressed in the same cell type, localized in the same cellular compartment, or expressed in the same developmental stage. These concerns, while quite relevant to a screen intended to elucidate natural biological functions of proteins, are entirely irrelevant to Applicants' methods. Applicants' system is intended to identify agents that activate TSA-responsive Sp3-mediated transcription, not to determine whether two proteins are naturally occurring "interacting partners." An agent identified in the presently claimed methods is potentially useful even if it does not occur naturally in any cell, much less meet the criteria of concern to Allen et al. In fact, the agents tested in the present methods need not even be proteins, and frequently will not be.

Thus, the concerns expressed by Allen et al. are not germane to the question of whether Applicants' methods would work as described.

Similar considerations apply to the Office's reliance on Fields and Sternglanz (TIG, 1994, 10:287-292). Like Allen et al., Fields and Sternglanz are cited for their teaching that association of two proteins in a two-hybrid screen does not necessarily indicate that the native proteins normally interact *in vivo*. Applicants' methods are not two-hybrid screens, do not rely on interactions between heterologous proteins, and produce valuable information that has nothing to do with how native proteins interact *in vivo*. The citation of Fields and Sternglanz is therefore inapt. It may be true, as the Office action states at page 7, that a "two-hybrid system is not predictably representative of the normal protein distribution or protein interactions found in cells even in cell culture," but since Applicants are not claiming a two-hybrid system and do not require results that are "representative of normal protein distribution or protein interactions," this statement is simply irrelevant to the issue of whether Applicants' methods will work.

The claimed methods provide a rapid and simple method to identify compounds that activate TSA-responsive Sp3-mediated transcription. One of skill in the art would appreciate that a screening method such as that presently claimed may produce some false positives; it would be no more than routine experimentation to perform further assays on any compounds identified by the present methods, to confirm the results of the initial assay. This is routine procedure in the art of identifying candidate pharmaceuticals and does not negate the usefulness of the assay as a quick means to screen large numbers of compounds. Furthermore, even if the methods do produce some false positives, the presence of some inoperative embodiments within the scope of a claim do not render the claim non-enabled. See, e.g., MPEP 2164.08(b). Finally, as the Federal Circuit noted in *In Re Brana*, "Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development." 51 F.3d 1560, 1568 (Fed. Cir. 1995). Applicants note again that the present claims are not drawn to identifying compounds for the treatment of cancer, but simply to methods of identifying agents that activate TSA-responsive Sp3-mediated transcription.

At pages 5-6, the Office Action states that "The term 'cell' as disclosed by the specification is not limited and encompasses both in vivo cells in intact hosts, as well as any in vitro cells in culture derived from any source and of any etiology." Applicants have amended claim 6 to specify that the cell used in the claimed method is a mammalian cell *in vitro*.

At pages 7-9, the Office Action cites Black et al., J. Cell. Physiol. 188:143-160, 2001, as proof that

> it could not be predicted that . . . an agent that activates TSA-responsive Sp3 mediated transcription using the claimed two-hybrid system could predictably identify an agent that activates TSA-responsive Sp3 mediated transcription either *in vivo* or *in vitro* cells or identify agents having cellular anti-proliferative or anticancer activity.

Applicants first point out for the record that Black et al. is not prior art to the present application, having been published after the present application's international priority date of March 23, 2000. To the extent that its teachings are still considered to be relevant to the question of whether applicants' claims are adequately enabled by the specification, applicants traverse on substantive grounds.

The Examiner's concerns appear to focus on Black et al.'s teachings about the "context-dependent" transcriptional properties of Sp3:

> Black et al teach that Sp3 has been found to activate or repress transcription, dependent on the cell line or promoter examined, meaning the transcriptional property of Sp3 is context-dependent (p. 145, col.1)....The teachings of Black et al indicate that the role of Sp3 in cell growth or growth inhibition is context-dependent and that many factors play a role in the cellular pathways involving 'Sp1 site'-containing promoters.

Even if one assumes that the Office Action's interpretation of Black et al.'s teachings is accurate, the Examiner's concerns are misplaced, because the teachings are simply irrelevant to the present claims. The claimed methods use a fusion protein containing a fragment of Sp3 that lacks critical parts of the zinc finger region, so lacks a functional DNA binding domain of Sp3. The DNA binding domain that is attached to that fragment is from a heterologous protein, not from Sp3. Thus, the sequence (or promoter) to which the fusion protein can bind is dictated not by the context-dependent Sp3 DNA binding domain that is naturally present in Sp3, and that was

studied by Black et al., but rather by the heterologous protein's DNA binding domain. One of ordinary skill would know to pick a DNA binding domain that will work in a predictable manner, i.e., one that has an identified target binding sequence that is not "context dependent". GAL4 is one well-known example that was utilized in Example 3 of the specification. In Example 3, a fusion protein containing a fragment of Sp3 combined with the GAL4 DNA binding domain was shown to work in that transcription of the reporter gene was increased in the presence of TSA. Since it worked with TSA, one of ordinary skill would be confident that the method would predictably identify agents that mimic TSA's role in activating Sp3. The person of ordinary skill would also realize that Black et al.'s teachings about the promoters to which Sp3 binds in the native cell, and "context" and "factors" relating to Sp3's natural "cellular pathways," are of no significance to the question of whether the claimed method works. In fact, the method is designed to avoid all those uncertainties. Thus, the Examiner's fear that "[a] high quantity of experimentation would be necessary to practice the invention as claimed" is simply without basis.

The Office action offers the following final remarks in support of the enablement rejection:

> Finally, those of skill in the art recognize that *in vitro* assays and or cell-cultured based assays are generally useful to observe basic physiological and cellular phenomenon such as screening the effects of potential drugs. However, clinical correlations are generally lacking. The greatly increased complexity of the *in vivo* environment as compared to the very narrowly defined and controlled conditions of an *in-vitro* assay does not permit a single extrapolation of *in vitro* assays to human diagnostic efficacy[1] with any reasonable degree of predictability.

The first line of the quoted text indicates that the Office has accepted that *in vitro* assays such as the method of the invention is generally useful for purposes such as drug screening. This position is consistent with past practice of the Office in regularly issuing patents claiming screening assays. It is also consistent with the law as interpreted by the courts (see, e.g., *In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995), *Cross v. Iizuka*, 753 F.2d 1040 (Fed. Cir. 1985), and

[1] Applicants are uncertain why the Examiner raises an issue regarding "human diagnostic efficacy" in the context of the present invention, which has nothing to do with diagnosis. To the extent this remains a concern of the Examiner's, she is asked to elaborate so that Applicants can respond appropriately.

Fujikawa v. Wattanasin, 93 F.3d 1559 (Fed. Cir. 1996), all of which point out the usefulness of *in vitro* screening assays as a first step in identifying biologically active compounds). Applicants are therefore puzzled as to the meaning of the apparently contradictory second sentence quoted above: "However, clinical correlations are generally lacking." If this is meant to be a characterization of screening assays in general (as implied by the word "generally"), it is neither true nor pertinent to the question of whether screening assays in general are patentable. As the Examiner is no doubt aware, screening assays are commonly patented even without evidence that agents identified in the screens have proven to be successful drugs. Generally speaking, i*n vitro* screens are extremely useful as a first pass to identify compounds that are potentially useful as drugs. In some cases, the screens may be validated using drugs with known clinical efficacy, but often such compounds do not exist. That does not mean the screens aren't useful. Regardless of whether the screens have been validated using known drugs, any compounds identified in the screen of course must be further tested in other types of screens, including *in vivo* tests, to confirm their usefulness. That simple fact does not mean the screens themselves are not generally useful—and patentable.

Even if the quoted statement about "clinical correlations" is instead meant to apply to the presently claimed methods in particular, it still is neither true nor pertinent. As noted above, the present methods are directed to methods of identifying agents that activate TSA-responsive Sp3-mediated transcription. There is no question that the methods will identify agents possessing that activity. Whether those agents eventually identified in the claimed assay will ultimately prove to be effective anticancer or antiproliferative compounds *per se* is irrelevant. Such an outcome is not a requirement of the claims and should not be read into them. Furthermore, even if a "clinical correlation" <u>were</u> required in order for a claimed screening assay to be patentable, such a "correlation" exists for the presently claimed methods. TSA is known to have *in vivo* efficacy as an antitumor agent (see discussion of that point above), and TSA gave a positive readout when tested using a method within the present claims. Thus, the "clinical correlation" has been made.

For at least these reasons, Applicants submit that the pending claims are amply enabled, and request withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

Conclusion

In light of the arguments and amendments made herein, Applicants submit that the pending claims are allowable and request early and favorable action thereon. If the Examiner feels that it would expedite allowance of the present application, she is invited to telephone the undersigned at (617) 956-5985.

No fees are believed to be due. Please apply any charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 14875-085001.

Respectfully submitted,

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